

5 Figure 6. Lovastatin concentration from the corresponding
wild-type *lovE* control is shown in matching fill pattern.
For example, *lovE* alleles 2, 7, 8 and 9 were all
transformed and assayed at the same time as the non-
hatched wild-type control. The horizontal line in each
10 individual box represents the median.

Lovastatin concentration was also determined by high
pressure liquid chromatography (HPLC). Briefly, 100 μ L of
broth sample was removed and diluted 1:10 into 70% H_2O -30%
acetonitrile (900 μ l). This mixture was spun down to
15 pellet debris at 13000 RPM for 5 minutes. 900 μ l of this
diluted broth was transferred to a vial and the sample was
analyzed by HPLC. 10 μ l were injected into a Waters HPLC
system (996 photo-diode array detector, 600 E pump
controller and 717 autosampler) equipped with a YMC-Pack
20 ODS column (Aq-302-3, 150 x 4.6 mm ID, S-3 μ m pore size)
and eluted with isocratic 40% aqueous acetic acid (0.7%)-
60% acetonitrile for 8 minutes. Lovastatin was detected
at 238 nm to have a retention time of 6.5 minutes and was
quantified using a calibration curve created from pure
25 lovastatin samples.

The results from ten individual transformants for
each *lovE* variant are shown in standard box plot format in
Figure 7A and 7B. Thirty individual wild-type *lovE*
transformants and ten individual MB2143 negative control
30 transformants were tested. Identical controls are plotted
in Figures 7A and 7B.

PCR analysis of *A. terreus* transformants demonstrates
that greater than fifty percent of the transformants
contain the transgene. Variability in levels of transgene
35 expression can presumably be influenced by integration
site and copy number. *lovE* variants containing identical
amino acid substitutions are labeled.

The amino acid and nucleic acid sequences of *lovE*
variant sequences are presented in Table 5 and Table 6,
40 respectively.